Preprint
UCRL-JC-144108

Glucose Recognition in Vitro Using Fluorescent Spectroscopy

G. Noronha, A. M. Heiss, J. R. Reilly, D. J. Vachon, Jr., D. R. Cary, N. P. Zaitseva, R. A. Reibold, S. M. Lane, T. A. Peyser, J. H. Satcher

This article was submitted to
222nd American Chemical Society National Meeting, Chicago, IL.,
August 26-30, 2001

U.S. Department of Energy
Lawrence Livermore National Laboratory

April 25, 2001

Glucose Recognition *In Vitro* Using Fluorescent Spectroscopy

G. Noronha, A.M. Heiss, J.R. Reilly, D.J. Vachon, Jr.
MiniMed Inc., 18000 Devonshire St., Northridge, CA 91325, aaron.heiss@minimed.com
D.R. Cary, N.P. Zaitseva, R.A. Reibold, S.M. Lane, T.A. Peyser, J.H. Satcher
Lawrence Livermore National Laboratory, Livermore, CA 94551

This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48 and under DOC NIST ATP Award Number 70NANB8H4065.

Diabetes is a disease that affects over 16 million people in the USA at a cost of 100 billion dollars annually.[1] The ability to regulate insulin delivery in people with Type 1 diabetes is imperative as is the need to manage glucose levels in all people with this disease. Our current method for monitoring glucose is a (FDA approved) minimally invasive enzymatic sensor that can measure glucose levels *in vivo* for three days. We are focused on developing a noninvasive implantable glucose sensor that will be interrogated by an external device. The material must be robust, easy to process, biocompatible and resistant to biofouling. In this Presentation we will discuss the development of a new polymeric matrix that can recognize physiological levels of glucose in vitro using fluorescent spectroscopy.

[1]American Diabetes Association, 1998. Economic consequences of diabetes mellitus in the U.S. in 1997. *Diabetes Care* **1998**, *21*, 296-299.

Glucose Recognition *In Vitro* Using Fluorescent Spectroscopy

G. Noronha, A.M. Heiss, J.R. Reilly, D.J. Vachon, Jr.
Minimed Inc., 18000 Devonshire St., Northridge, CA 91325, aaron.heiss@minimed.com
D.R. Cary, N.P. Zaitseva, R.A. Reibold, S.M. Lane, T.A. Peyser, J.H. Satcher
Lawrence Livermore National Laboratory, Livermore, CA 94551

This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48 and under DOC NIST ATP Award Number 70NANB8H4065.

I. Introduction

Diabetes is a major debilitating disease with 16 million diabetics in the US alone, a number that has recently been predicted to be increasing in an alarming fashion rather than following some steady far more comfortable growth trend. Of these 16 million, roughly ten percent are type I diabetics. A variety of approaches towards tackling the disease have been employed, but to date, there has been no replacement for simply supplying insulin in some timely fashion. The second part of this problem, and arguably the second half of the equation is determining the amount of glucose since it is the amount of glucose that must finally be regulated. While insulin can be delivered via injections, or via a pump, a glucose sensor suitable for use in the artificial pancreas must be accurate over the long term, capable of frequent or continuous sampling, and biocompatible. It should also be inexpensive and easy to calibrate. Research into blood glucose sensors initially concentrated on enzymatic devices but has recently focused on noninvasive optical methods. Because of the potential market for a successful sensor, much of the research is proprietary, with claims exceeding scientific data. Several sensor types are currently being studied.

The ability to sense glucose by some relatively simple and reproducible method is a crucial objective to some of the longer term programs at MiniMed, wherein continuous monitoring of blood glucose over several weeks, months or perhaps years, by a single implantable device would be exceptionally kind to a person that needs to monitor blood glucose. As of now, the most common method of sensing glucose is through the good graces of glucose oxidase, whose exceptional abilities in sensing glucose are offset by its limited stability, making it not viable after a few days. One solution to this problem is to use a chemical method of detecting the glucose, the recognition aided of course by a measurable change of some kind. Analyte detection by fluorescence techniques is quite popular since micro molar amounts of fluorophore can be used, and it is quite easy to measure comparable amounts of substrate, and the detection means are well understood. The use of boronates as the business end of sugar recognition dates back around 40 years to the work of Lorand, but has more recently been popularized by several groups including those of Czarnik, da Silva and perhaps most extensively, by Shinkai.

A device incorporating such a recognition element as the "sensor" would be incorporated into a polymer matrix, which would have to be biocompatible. A suitable means of excitation and detection of the fluorescence changes would be needed to complete the device. Whether the transmission would be detected through the skin, or amplified suitably, converted to a different wavelength, or aided at some point by fiber optic means, if not some combination of these ideas, is not limiting since the key issue at this point in the program is to be able to present a simple to synthesize, stable and reproducible sensing molecule in a biocompatible (polymer) matrix. Since the fluorophores are most commonly fairly hydrophobic organic molecules, one means of possibly improving water solubility would be to incorporate hydrophillic units into the polymer matrix. Several designs present themselves, including the use of block copolymers incorporating other water compatible blocks, fluorophores directly attached to water compatible, random copolymers with hydrophilic monomer, or by the use of modifying naturally occurring hydrogels (*e.g.*, gelatin).

As part of this program, one of the starting objectives is to build polymeric units containing fluorophore, and polymer with aqueous and bio-compatibility, and demonstrate the ability of these units to tranduce physiological levels of glucose in water (buffered, pH = 7.4) in an accurate and reproducible fashion.

Our own pursuit of a sensing device involves (1) establishing physiological applicable glucose transduction reference data, (2) building more hydrophilic polymer units, (3) establishing physiological transduction capabilities of such units, and (4) and polymeric units containing fluorophores possessing aqueous tolerance and bio-compatibility are built, then it left to demonstrate the ability of these units to tranduce physiological levels of glucose in water in reproducible fashion with good precision, accuracy and good sensing abilities.

II. Experimental

Development of standard data for the fluorescence studies.

It is not obvious what kind of fluorescence will be seen in aqueous solutions since most of the related literature data is in mixtures of methanol and water. In addition, we needed a set a lower limit for how much glucose we would be able to detect, that is, what kind of response would we get at physiological glucose concentrations. All experiments are run at room temperature (ca 20°C). Wherever reference is made to water or aqueous solutions, it is phosphate buffered saline (PBS) at pH = 7.4. In all cases [Fluorophore] = 10 μM.

Fluorophore studies

Several fluorescence experiments have now established the superior nature of our glucose sensing fluorophore under aqueous conditions. Sensing at concentrations of 100 nM is exceptionally good, and along with a series of dilution and sensing

experiments, we have shown that lower concentrations of fluorophore are in fact, excellent in terms of detecting and transducing glucose. Thus, it may be favorable (perhaps essential) to use lower fluorophore concentrations, *i.e.*, 10 μM or less.

Solution studies

Solution studies in aq PBS at pH 7.4 are used for getting a good understanding of the basic system and the associated problems.

MeOH spikes compared to DMSO spikes

We are using as a basis for the transductions obtained with our fluorophore incorporated in polymer films the homogeneous solution transductions of our fluorophore in aqueous PBS at pH 7.4. Our fluorophore is hydrophobic and therefore insoluble in water. It is fairly common in the literature to use a fairly large amount (between 33% and 75%) of MeOH as means to solubilize our fluorophore in water. We showed recently that results obtained under these conditions are not physiologically applicable since (a) results tended to be typically much better in the solutions with large amounts of MeOH; (b) sometimes results obtained in such combinations were not at all reproducible under physiological conditions; and (c) MeOH evaporation from solutions caused unnecessary changes in florescence intensities as an added complication. A careful study showed us that we could obtain solution data with far smaller amounts of "spike" solvent. To this end, both MeOH and DMSO were used in small amounts, that is, usually around 0.5% or less. It was implicitly assumed that data under these conditions might be similar but since the small amount of spike solvent still might make a difference, this still remained as an issue.

A systematic study was carried out to compare MeOH and DMSO as "spike" solvents under similar conditions. One comparative set of data is shown in **Figure 1** below, wherein our fluorophore concentration is maintained at 2.5 μM. The blue curves (lower two) are obtained with DMSO spikes while the pink curves (upper two) are those obtained with MeOH spikes. In each case the top curve is the amplification obtained with 200 mg/dL glucose. Therefore, the two blue curves can be compared and the two pink curves can be compared and of course, the blue curves ought to be compared to the pink curves.

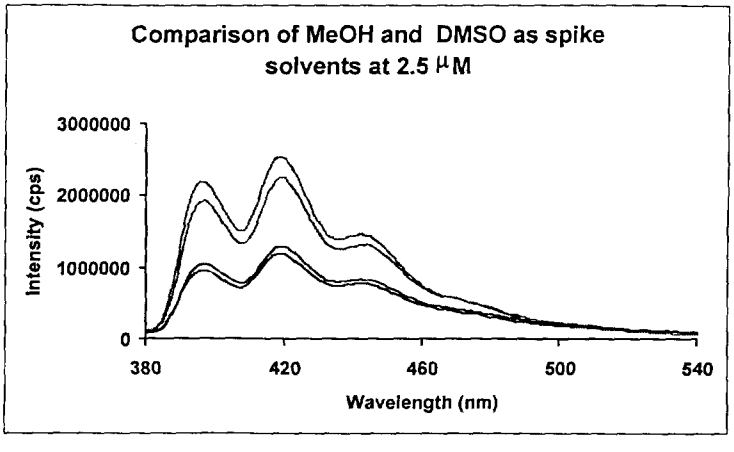


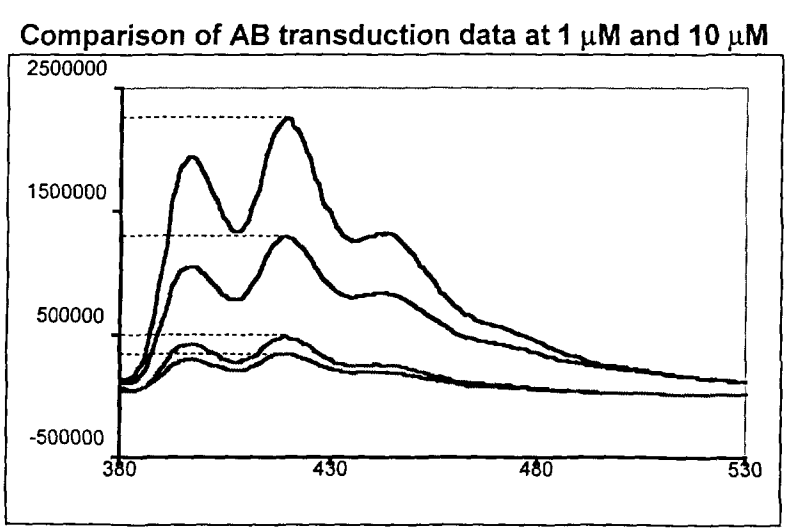
Figure 1. A comparison of solution fluorescence of AB in aq. PBS at pH 7.4. The pink cuves (upper two) result when MeOH is used as the solvent to make the stock AB solution used for spiking, while the blue curves are those obtained when DMSO is used. In each of the cases, the higher intensity curve is with the amplification obtained with 200 mg/dL glucose.

Two immediate conclusions are first, MeOH as a spike solvent changes the position of the baseline, and second, the transduction with MeOH as spike solvent is also increased compared to the optical amplification using DMSO. For example, the transduction at 200 mg/dL glucose is between 70% and 80% using DMSO as spike solvent, but more than 125% using MeOH as spike solvent. In addition partly on account of the fact that such a small amount of MeOH easily evaporates, our fluorophore containing solutions made with MeOH spikes seem to form precipitates quite readily leading to large changes in results over a short period of time. For all of these reasons detailed above, it seems better to use DMSO as spike solvent for carrying out studies of our fluorophore in aq. PBS at pH 7.4.

DMSO spikes at different DMSO concentrations.

One of the immediate tests to perform when using DMSO as a spike solvent is to test whether changes in the concentration of DMSO make a difference to the baseline or to the transduction. Although data is not provided here, a ten-fold change in DMSO concentration (0.1% to 10 %), showed no apparent difference in either the baseline or in the transduction at 200 mg/dL glucose. First, if DMSO has an effect, then that effect is not in play in the range that is used when DMSO is used as a spike solvent. If the effect is really strong at a low concentration of DMSO (far below that used in these experiments), then we are operating in a range in which the saturation has occurred and thus there is no apparent farther manifestation of DMSO being a problem. In case much higher concentrations of DMSO are required, that too is not an issue, since such concentrations are far beyond what is used in the spike. Second, within the framework of its use as a spike solvent, the amount of DMSO to not be of any consequence to the transduction studies.

DMSO spikes with different AB concentrations.



The effect of the concentration on the fluorophore was the next to be tested. With fluorophore concentration at 1 μM and 10 μM, spectra were run without any glucose and with 200 mg/dL glucose for each of the concentrations. The data is shown in **Figure 2.**

Figure 2.
Fluorophore transduction data in aq. PBS at pH 7.4 at two different concentrations; 1 μM (lower two curves, pink) and 10 μM (upper two curves, blue) showing signal amplification for each of the two cases. The 10 μM solution gives better transduction (*ca.* 75% at 200 mg/dL glucose).

Within the range of concentrations studied, the higher the fluorophore concentration, the better the transduction. For example, the transduction obtained for the 10 μM fluorophore solution is *ca.* 75% at 200 mg/dL glucose (upper two curves) while that obtained at 1 μM is much lower although not lower by a factor of 10. These results have significant implications for the concentration of the fluorophore to be used in a polymeric system. It may be likely that a higher amount of fluorophore is better, although the actual amount to be used would need to be optimized.

Polymer Studies

Our fluorophore has been incorporated into a polymeric matrix that produces reasonable transduction of aqueous solutions of glucose *in vitro*. Several studies have been done to determine the optimal loading of fluorophore, choice of materials, method of polymerization and processing. Films cast of our material transduce glucose *ca.* 10% at 200 mg/dL. Response is linear from 50-400 mg/dL.

Conclusions

The fluorophore

We use our fluorophore as a model in aq. PBS at pH 7.4, and although we had to use a tiny amount of MeOH or DMSO for solubility (0.2 % is the smallest we have used), we now know that our fluorophore transduces glucose quite well (*ca.* 70% - 80% at 200 mg/dL glucose using DMSO as the spike solvent) under aqueous physiological conditions.

Numerous and sundry factors affect this number. Thus getting basic data as a starting point for *in vitro* calibrations is both difficult and complicated. We have now begun to have a good enough understanding of the workings of this basic system and may capitalize on this in a rational manner.

As a "spike" solvent DMSO is better than MeOH for reasons detailed in the report.

The concentration of the fluorophore in solution makes a difference. The higher the amount of [fluorophore], the better the transduction. This has implications for the concentration of fluorophore to be used in a polymer matrix.

Future Work

Calibration of this polymer *in vitro* is essential to find out the limits of detection and the error on the response.

We will then test reproducibility, reversibility and life of the system, that is, how many times is it possible to cycle back and forth.